REMARKS

The specification is amended to append a copy of the Sequence Listing in accord with 37 CFR 1.821(c). Applicants enclose with the amendment a CRF copy of the Sequence Listing and a declaration under 37 CFR 1.812.

The specification has been amended to include sequence identifier numbers where appropriate. A marked-up version of each amended section is appended hereto.

This application is a continuation-in-part application of application number 09/500,135, filed Feb.8, 2000. With the entry of the instant amendment, claims 1, 4 - 13, 29 and 30 are pending. The pending claims are directed to a variant protein having an altered immunogenic response as compared to a precursor protein wherein the variant protein produces a greater immunogenic response. Claims 29 and 30 have been added by the amendment and do not introduce new matter. Claim 29 is directed to therapeutic proteins and support is found at page 23, line 7 of the specification. Claim 30 is a new independent claim direct to the way in which the T-cell epitope is altered and support is found in the original claims. Original claims 2, 3 and 14 - 28 have been canceled.

Respectfully submitted,

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MARKED-UP VERSION OF EACH AMENDED SECTION OF THE SPECIFICATION

(Changes are indicated in bold with deletions indicated as brackets and additions underlined)

(2) BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 A, B1, B2 and B3 illustrate the DNA (SEQ ID**[:]** NO**:** 1) and amino acid (SEQ ID**[:]** NO**:** 2) sequence for *Bacillus amyloliquefaciens* subtilisin (BPN') and a partial restriction map of this gene.

Fig. 2 illustrates the conserved amino acid residues among subtilisins from *Bacillus amyloliquefaciens* **[(SEQ ID:NO 3)]** and *Bacillus lentus* (wild-type) **[(SEQ ID:NO 4)]**.

Figs. 3A and 3B illustrate an amino acid sequence alignment of subtilisin type proteases from *Bacillus amyloliquefaciens* (BPN') **(SEQ ID NO: 3)**, *Bacillus subtilis* **(SEQ ID NO: 4)**, *Bacillus licheniformis* (SEQ ID**[:]** NO**:** 5) and *Bacillus lentus* **(SEQ ID NO: 6)**. The symbol * denotes the absence of specific amino acid residues as compared to subtilisin BPN' **(SEQ ID NO: 3)**.

Fig. 4 illustrates the additive T-cell response of 16 peripheral mononuclear blood samples to peptides corresponding to the *Bacillus lentus* protease (GG36). Peptide E05 includes the region comprising residues corresponding to 170-173 in protease from *Bacillus amyloliquefaciens*.

Fig. 5 illustrates the additive T-cell response of 10 peripheral mononuclear blood samples to peptides corresponding to the human subtilisin molecule. Peptides F10, F9, F8 and F7 all contain the amino acid sequence DQMD corresponding to the region comprising residues corresponding to 170-173 in protease from *Bacillus amyloliquefaciens* **(SEQ ID NO: 3)** in the sequence alignment of Fig. 3.

Fig. 6A and 6B/6C illustrate amino acid strings **(SEQ ID NOS: 7 through 207)** corresponding to peptides derived from the sequence of *Bacillus lentus* protease and a human subtilisin, respectively.

Fig. 7 illustrates the amino acid sequence of human subtilisin **[(SEQ ID:NO 6)]** **SEQ ID NO: 208**.

Fig. 8 illustrates an amino acid sequence alignment of BPN' (*Bacillus amyloliquefaciens*) **(SEQ ID NO: 3)** protease, SAVINASE (*Bacillus lentus*) **(SEQ ID NO: 6)** protease and human subtilisin (S2HSBT) **(SEQ ID NO: 209)**.

Fig. 9 illustrates the T-cell response to peptides derived from *Bacillus lentus* protease in a sample taken from an individual known to be hypersensitive to *Bacillus lentus* protease. Peptide E05 represents the region corresponding to 170-173 in protease from *Bacillus amyloliquefaciens.*

Fig. 10 illustrates the T-cell response to various alanine substitutions in the E05 *Bacillus lentus* protease peptide set in a sample taken from an individual known to be hypersensitive to *Bacillus lentus* protease.

Fig. 11 illustrates the T-cell response to various alanine substitutions in the E05 protease peptide (an embodiment of the T-cell epitope designated unmodified sequence) set in a sample taken from an individual known to be hypersensitive to the protease; the sequences for each peptide **(SEQ ID NOS: 210 through 221)** are also shown.

Fig. 12 illustrates the percent responders to the human subtilisin molecule.

Fig. 13A illustrates the T-cell response of peptides derived from *Humicola insolens* endogluconase (Accession number A23635). Peptides A02 **(SEQ ID NO: 222)** and F06 **(SEQ ID NO: 223)** represent the region corresponding to residues 70-84 and 37-51, respectively, embodiments of the T-cell epitope, of *Humicola insolens* endogluconase **(SEQ ID NO: 224)** , wherein the full length sequence is shown in Fig.13B and A02 **(SEQ ID NO: 222)** and F06 **(SEQ ID NO: 223)** are shown underlined and in bold.

Fig. 14A illustrates the T-cell response to peptides derived from *Thermomyces lanuginosa* lipase (Accession number AAC08588 and PID number g2997733). Peptides B02 **(SEQ ID NO: 225)** and C06 **(SEQ ID NO: 226)** represent the regions corresponding to residues 83-100 and 108-121, respectively, embodiments of the T-cell epitope, of *Thermomyces lanuginosa* lipase **(SEQ ID NO: 227)**, wherein the full length sequence is shown in Fig.14B and B02 **(SEQ ID NO: 225)** and C06 **(SEQ ID NO: 226)** are shown underlined and in bold.

Fig. 15A illustrates the T-cell response to peptides derived from *Streptomyces plicatus* endo-beta-N-acetylglucosaminidase. (Accession number P04067). Peptide C06 **(SEQ ID NO: 228)** represents the region corresponding to residues 126-140, an embodiment of the T-cell epitope, of *Streptomyces plicatus* endo-beta-N-acetylglucosaminidase **(SEQ ID NO: 229)**, wherein the full length sequence is shown in Fig.15B and C06 **(SEQ ID NO: 228)** is shown underlined and in bold.

Fig. 16 illustrates the T-cell response to peptides derived from BPN' compiled for 22 individuals, wherein the sequences of preferred T-cell epitopes **(SEQ ID NO: 230 through 231)** are indicated.

Fig. 17 illustrates the T-cell response to peptides derived from GG36 compiled for 22 individuals, wherein the sequences of embodiments of T-cell epitopes **(SEQ ID NO: 232 through 235)** are indicated, GSISYPARYANAMAVGA **(SEQ ID NO: 234)** and GAGLDIVAPGVNVQS **(SEQ ID NO: 235)** being preferred.

Fig. 18 is an embodiment of a hybrid protein **(SEQ ID NO: 236)** provided herein, where the N-terminus comprises N-terminal GG36 sequence and the C-terminus comprises C-terminal BPN' sequence, and wherein a comparison of the sequences with those shown in Fig. 8 indicates that the hybrid formed omits preferred T-cell epitopes of each protein.

Figure 19 is a comparison of ELISA titers for *B. amyloliquefaciens* subtilisin and the same subtilisin but engineered to contain a T-cell epitope from *B.* **[*lentis*] *lentus*** subtilisin. Figure 19a represents the titer at 4 weeks; Figure 19b at 6 weeks, Figure 19c at 8 weeks and Figure 19d at 10 weeks.

Figure 20 is a time course study of ELISA titers for *B. amyloliquefaciens* subtilisin and the same subtilisin but engineered to contain a T-cell epitope from *B.* **[*lentis*] *lentus*** subtilisin. Figure 20a represents the titer for a 1µg dose of enzyme, Figure 20b a 5 µg dose and Figure 20c a 20 µg dose. - -

(3) Paragraph at page 12, line 33 through page 13, line 2.

"Human subtilisin" means proteins of human origin which have subtilisin type catalytic activity, e.g., the kexin family of human derived proteases. An example of such a protein is represented by the sequence in Fig. 7 **(SEQ ID NO: 208)**. Additionally, derivatives or homologs of proteins provided herein, including those from non-human sources such as mouse or rabbit, which retain the essential activity of the peptide, such as the ability to hydrolyze peptide bonds, etc., have at least 50%, preferably at least 65% and most preferably at least 80%, more preferably at least 90%, and sometimes as much as 95 or 98% homology to the polypeptide of interest. In one embodiment, the polypeptide of interest is shown in the Figures.

(4) Section starting at page 13, line 34 through page 14, line 12.

These conserved residues, thus, may be used to define the corresponding equivalent amino acid residues of *Bacillus amyloliquefaciens* subtilisin in other subtilisins such as subtilisin from *Bacillus lentus* (PCT Publication No. W089/06279 published July 13, 1989), the preferred protease precursor enzyme herein, or the subtilisin referred to as PB92 (EP 0 328 299), which is highly homologous to the preferred *Bacillus lentus* subtilisin. The amino acid sequences of certain of these subtilisins are aligned in Figs. 3A and 3B **(SEQ ID NOS: 3 - 6)** with the

sequence of *Bacillus amyloliquefaciens* **(SEQ ID NO: 3)** subtilisin to produce the maximum homology of conserved residues. As can be seen, there are a number of deletions in the sequence of *Bacillus lentus* **(SEQ ID NO: 6)** as compared to *Bacillus amyloliquefaciens* **(SEQ ID NO: 3)** subtilisin. Thus, for example, the equivalent amino acid for Val165 in *Bacillus amyloliquefaciens* **(SEQ ID NO: 3)** subtilisin in the other subtilisins is isoleucine for *B. lentus* **(SEQ ID NO: 6)** and *B. licheniformis* **(SEQ ID NO: 5)**.

(5) The second full paragraph of Example 2 at page 25, line 23 through line 29.

Peptides used correspond to amino acid residue strings in *Bacillus lentus* as provided in Figure 8 **(SEQ ID NO: 6)**, and peptides correspond to amino acid residues in human subtilisin as provided in Figure 7 **(SEQ ID NO: 208)**. The peptides used corresponding to the proteases is provided in Fig. 6 **(SEQ ID NOS: 7 - 207)**. All tests were performed at least in duplicate. All tests reported displayed robust positive control responses to the antigen tetanus toxoid. Responses were averaged within each experiment, then normalized to the baseline response. A positive event was recorded if the response was at least 3 times the baseline response.

(6) The sixth full paragraph of Example 2 at page 26, line 13 through line 22.

Fig. 10 shows the T-cell response to various alanine substitutions in the E05 peptide derived from *Bacillus lentus* protease in a sample taken from an individual known to be hypersensitive to *Bacillus lentus* protease. Alanine substitutions were used as substitutions for the purpose of determining the role of any specific residue within the epitope. The legend of Figure 10 refers to the position of the peptide in which an alanine was substituted, i.e., in peptide E06 (sequence GSISYPARYANAMAV **(SEQ ID NO: 210)**), G to A = 2, S to A = 3, I to A = 4, S to A = 5, Y to A = 6, P to A = 7, R to A = 8, Y to A = 9, N to A = 10, M to A = 11 and V to A = 12. As indicated in Figure 10, substitution of either of the residues R170A, Y171A and/or N173A in protease from *Bacillus lentus* results in dramatically reduced response in the hypersensitive individual's blood sample.

(7) Example 6 at page 27.

Example 6

Identification of T-Cell Epitopes in a Protease Hybrid (GG36-BPN') **(SEQ ID NO: 236)**

After determining the location of a T-cell epitope, a protease hybrid was constructed using established protein engineering techniques. The hybrid was constructed so that a highly

allergenic amino acid sequence of the protein was replaced with a corresponding sequence from a less allergenic homolog. In this instance, the first 122 amino acids of the protease were derived from GG36, and the remaining amino acid sequence was derived from BPN'.

The hybrid was first tested from a 100 ppm sample in North American condition in 24 well assay at .5 ppm, superfixed swatches, liquid (Tide KT) at .5 in 24 well assay with 3K swatches, and in the N'N'-dimethyl Casein Assay, 5 g/l DMC in NA detergent, TNBS dectection method.

The results are shown in Figures 16 **(SEQ ID NOS: 230 and 231)**, 17 **(SEQ ID NOS: 232 through 235)** and 18 **(SEQ ID NO: 236)**.

MARKED-UP VERSION OF AMENDED CLAIMS:

1.(Once Amended) A variant of a polypeptide of interest comprising a T-cell epitope, wherein said variant differs from said polypeptide of interest by having an altered T-cell epitope such that said variant [and said polypeptide produce different immunogenic responses] produces an immunogenic response in an individual which is greater than the immunogenic response produced by said polypeptide of interest .

2. Canceled

3. Canceled

8.(Once amended) The variant of claim 1 wherein said T-cell epitope is altered by having a terminal portion of said polypeptide of interest comprising said T-cell epitope replaced with a corresponding terminal portion of a homolog of said polypeptide of interest wherein said homolog does not comprise a T-cell [cell] epitope identical to said replaced T-cell epitope.

Claims 14 - 28 canceled